

What is claimed is:

1. A method for sequencing a nucleic acid by detecting the identity of a nucleotide analogue after the nucleotide analogue is incorporated into a growing strand of DNA in a polymerase reaction, which comprises the following steps:
  - (i) attaching a 5' end of the nucleic acid to a solid surface;
  - (ii) attaching a primer to the nucleic acid attached to the solid surface;
  - (iii) adding a polymerase and one or more different nucleotide analogues to the nucleic acid to thereby incorporate a nucleotide analogue into the growing strand of DNA, wherein the incorporated nucleotide analogue terminates the polymerase reaction and wherein each different nucleotide analogue comprises (a) a base selected from the group consisting of adenine, guanine, cytosine, thymine, and uracil, and their analogues; (b) a unique label attached through a cleavable linker to the base or to an analogue of the base; (c) a deoxyribose; and (d) a cleavable chemical group to cap an -OH group at a 3'-position of the deoxyribose;
  - (iv) washing the solid surface to remove unincorporated nucleotide analogues;

- 5 (v) detecting the unique label attached to the nucleotide analogue that has been incorporated into the growing strand of DNA, so as to thereby identify the incorporated nucleotide analogue;
- 10 (vi) adding one or more chemical compounds to permanently cap any unreacted -OH group on the primer attached to the nucleic acid or on a primer extension strand formed by adding one or more nucleotides or nucleotide analogues to the primer;
- 15 (vii) cleaving the cleavable linker between the nucleotide analogue that was incorporated into the growing strand of DNA and the unique label;
- 20 (viii) cleaving the cleavable chemical group capping the -OH group at the 3'-position of the deoxyribose to uncap the -OH group, and washing the solid surface to remove cleaved compounds; and
- 25 (ix) repeating steps (iii) through (viii) so as to detect the identity of a newly incorporated nucleotide analogue into the growing strand of DNA;
- 30

wherein if the unique label is a dye, the order of steps (v) through (vii) is: (v), (vi), and (vii); and

5            wherein if the unique label is a mass tag, the order of steps (v) through (vii) is: (vi), (vii), and (v).

10           2.    The method of claim 1, wherein the solid surface is glass, silicon, or gold.

            3.    The method of claim 1, wherein the solid surface is a magnetic bead, a chip, a channel in a chip, or a porous channel in a chip.

15           4.    The method of claim 1, wherein the step of attaching the nucleic acid to the solid surface comprises:

20           (i)    coating the solid surface with a phosphine moiety,

            (ii) attaching an azido group to the 5' end of the nucleic acid, and

25           (iii) immobilizing the 5' end of the nucleic acid to the solid surface through interaction between the phosphine moiety on the solid surface and the azido group on the 5' end of  
30           the nucleic acid.

5. The method of claim 4, wherein the step of coating the solid surface with the phosphine moiety comprises:

- 5 (i) coating the surface with a primary amine, and
- (ii) covalently coupling a N-hydroxysuccinimidyl ester of triarylphosphine with the primary amine.

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6. The method of claim 1, wherein the nucleic acid that is attached to the solid surface is a single-stranded DNA.

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7. The method of claim 1, wherein the nucleic acid that is attached to the solid surface in step (i) is a double-stranded DNA, wherein only one strand is directly attached to the solid surface, and wherein the strand that is not directly attached to the solid surface is removed by denaturing before proceeding to step (ii).

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8. The method of claim 1, wherein the nucleic acid that is attached to the solid surface is a RNA, and the polymerase in step (iii) is reverse transcriptase.

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9. The method of claim 1, wherein the primer is attached to a 3' end of the nucleic acid in step (ii) and wherein the attached primer comprises a stable loop and an -OH group at a 3'-position of a

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deoxyribose capable of self-priming in the polymerase reaction.

- 5 10. The method of claim 1, wherein the step of attaching the primer to the nucleic acid comprises hybridizing the primer to the nucleic acid or ligating the primer to the nucleic acid.
- 10 11. The method of claim 1, wherein one or more of four different nucleotide analogues is added in step (iii), wherein each different nucleotide analogue comprises a different base selected from the group consisting of thymine or uracil or an analogue of thymine or uracil, adenine or an analogue of adenine,  
15 cytosine or an analogue of cytosine, and guanine or an analogue of guanine, and wherein each of the four different nucleotide analogues comprises a unique label.
- 20 12. The method of claim 1, wherein the cleavable chemical group that caps the -OH group at the 3'-position of the deoxyribose in the nucleotide analogue is  $-\text{CH}_2\text{OCH}_3$  or  $-\text{CH}_2\text{CH}=\text{CH}_2$ .
- 25 13. The method of claim 1, wherein the unique label that is attached to the nucleotide analogue is a fluorescent moiety or a fluorescent semiconductor crystal.
- 30 14. The method of claim 13, wherein the fluorescent moiety is selected from the group consisting of 5-carboxyfluorescein, 6-carboxyrhodamine-6G,

N,N,N',N'-tetramethyl-6-carboxyrhodamine, and 6-carboxy-X-rhodamine.

15. The method of claim 1, wherein the unique label  
5 that is attached to the nucleotide analogue is a fluorescence energy transfer tag which comprises an energy transfer donor and an energy transfer acceptor.
- 10 16. The method of claim 15, wherein the energy transfer donor is 5-carboxyfluorescein or cyanine, and wherein the energy transfer acceptor is selected from the group consisting of  
15 dichlorocarboxyfluorescein, dichloro-6-carboxyrhodamine-6G, dichloro-N,N,N',N'-tetramethyl-6-carboxyrhodamine, and dichloro-6-carboxy-X-rhodamine.
17. The method of claim 1, wherein the unique label  
20 that is attached to the nucleotide analogue is a mass tag that can be detected and differentiated by a mass spectrometer.
18. The method of claim 17, wherein the mass tag is  
25 selected from the group consisting of a 2-nitro- $\alpha$ -methyl-benzyl group, a 2-nitro- $\alpha$ -methyl-3-fluorobenzyl group, a 2-nitro- $\alpha$ -methyl-3,4-difluorobenzyl group, and a 2-nitro- $\alpha$ -methyl-3,4-dimethoxybenzyl group.
- 30 19. The method of claim 1, wherein the unique label is attached through a cleavable linker to a 5-position

of cytosine or thymine or to a 7-position of deaza-adenine or deaza-guanine.

- 5           20. The method of claim 1, wherein the cleavable linker between the unique label and the nucleotide analogue is cleaved by a means selected from the group consisting of one or more of a physical means, a chemical means, a physical chemical means, heat, and light.
- 10           21. The method of claim 20, wherein the cleavable linker is a photocleavable linker which comprises a 2-nitrobenzyl moiety.
- 15           22. The method of claim 1, wherein the cleavable chemical group used to cap the -OH group at the 3'-position of the deoxyribose is cleaved by a means selected from the group consisting of one or more of a physical means, a chemical means, a physical chemical means, heat, and light.
- 20           23. The method of claim 1, wherein the chemical compounds added in step (vi) to permanently cap any unreacted -OH group on the primer attached to the nucleic acid or on the primer extension strand are a polymerase and one or more different dideoxynucleotides or analogues of dideoxynucleotides.
- 25           24. The method of claim 23, wherein the different dideoxynucleotides are selected from the group consisting of 2',3'-dideoxyadenosine 5'-
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triphosphate, 2',3'-dideoxyguanosine 5'-  
triphosphate, 2',3'-dideoxycytidine 5'-  
triphosphate, 2',3'-dideoxythymidine 5'-  
triphosphate, 2',3'-dideoxyuridine 5'-triphosphate,  
5 and their analogues.

25. The method of claim 1, wherein a polymerase and one  
or more of four different dideoxynucleotides are  
added in step (vi), and wherein each different  
10 dideoxynucleotide is selected from the group  
consisting of 2',3'-dideoxyadenosine 5'-  
triphosphate or an analogue of 2',3'-  
dideoxyadenosine 5'-triphosphate; 2',3'-  
dideoxyguanosine 5'-triphosphate or an analogue of  
15 2',3'-dideoxyguanosine 5'-triphosphate; 2',3'-  
dideoxycytidine 5'-triphosphate or an analogue of  
2',3'-dideoxycytidine 5'-triphosphate; and 2',3'-  
dideoxythymidine 5'-triphosphate or 2',3'-  
dideoxyuridine 5'-triphosphate or an analogue of  
20 2',3'-dideoxythymidine 5'-triphosphate or an  
analogue of 2',3'-dideoxyuridine 5'-triphosphate.

26. The method of claim 17, wherein the mass tag is  
detected using a parallel mass spectrometry system  
25 which comprises a plurality of atmospheric pressure  
chemical ionization mass spectrometers for parallel  
analysis of a plurality of samples comprising mass  
tags.

30 27. A method of simultaneously sequencing a plurality  
of different nucleic acids, which comprises



simultaneously applying the method of claim 1 to the plurality of different nucleic acids.

5           28. Use of the method of claim 1 or 27 for detection of  
single nucleotide polymorphisms, genetic mutation  
analysis, serial analysis of gene expression, gene  
expression analysis, identification in forensics,  
genetic disease association studies, DNA  
sequencing, genomic sequencing, translational  
10 analysis, or transcriptional analysis.

29. A method of attaching a nucleic acid to a solid  
surface which comprises:

15           (i) coating the solid surface with a phosphine  
moiety,

            (ii) attaching an azido group to a 5' end of the  
nucleic acid, and

20           (iii) immobilizing the 5' end of the nucleic acid  
to the solid surface through interaction  
between the phosphine moiety on the solid  
surface and the azido group on the 5' end of  
25 the nucleic acid.

30. The method of claim 29, wherein the step of coating  
the solid surface with the phosphine moiety  
comprises:

30           (i) coating the surface with a primary amine,  
and

- (ii) covalently coupling a N-hydroxysuccinimidyl ester of triarylphosphine with the primary amine.

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31. The method of claim 29, wherein the solid surface is glass, silicon, or gold.

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32. The method of claim 29, wherein the solid surface is a magnetic bead, a chip, a channel in a chip, or a porous channel in a chip.

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33. The method of claim 29, wherein the nucleic acid that is attached to the solid surface is a single-stranded DNA, a double-stranded DNA or a RNA.

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34. The method of claim 33, wherein the nucleic acid is a double-stranded DNA and only one strand is attached to the solid surface.

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35. The method of claim 34, wherein the strand of the double-stranded DNA that is not attached to the solid surface is removed by denaturing.

36. Use of the method of claim 29 for gene expression analysis, microarray based gene expression analysis, mutation detection, translational analysis, or transcriptional analysis.

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37. A nucleotide analogue which comprises:

(a) a base selected from the group consisting of adenine or an analogue of adenine, cytosine or an analogue of cytosine, guanine or an analogue of guanine, thymine or an analogue of thymine, and uracil or an analogue of uracil;

(b) a unique label attached through a cleavable linker to the base or to an analogue of the base;

(c) a deoxyribose; and

(d) a cleavable chemical group to cap an -OH group at a 3'-position of the deoxyribose.

38. The nucleotide analogue of claim 37, wherein the cleavable chemical group that caps the -OH group at the 3'-position of the deoxyribose is  $-\text{CH}_2\text{OCH}_3$  or  $-\text{CH}_2\text{CH}=\text{CH}_2$ .

39. The nucleotide analogue of claim 37, wherein the unique label is a fluorescent moiety or a fluorescent semiconductor crystal.

40. The nucleotide analogue of claim 39, wherein the fluorescent moiety is selected from the group consisting of 5-carboxyfluorescein, 6-carboxyrhodamine-6G, N,N,N',N'-tetramethyl-6-carboxyrhodamine, and 6-carboxy-X-rhodamine.

41. The nucleotide analogue of claim 37, wherein the unique label is a fluorescence energy transfer tag

which comprises an energy transfer donor and an energy transfer acceptor.

- 5 42. The nucleotide analogue of claim 41, wherein the energy transfer donor is 5-carboxyfluorescein or cyanine, and wherein the energy transfer acceptor is selected from the group consisting of dichlorocarboxyfluorescein, dichloro-6-carboxyrhodamine-6G, dichloro-N,N,N',N'-tetramethyl-6-carboxyrhodamine, and dichloro-6-carboxy-X-rhodamine.
- 10
- 15 43. The nucleotide analogue of claim 37, wherein the unique label is a mass tag that can be detected and differentiated by a mass spectrometer.
- 20 44. The nucleotide analogue of claim 43, wherein the mass tag is selected from the group consisting of a 2-nitro- $\alpha$ -methyl-benzyl group, a 2-nitro- $\alpha$ -methyl-3-fluorobenzyl group, a 2-nitro- $\alpha$ -methyl-3,4-difluorobenzyl group, and a 2-nitro- $\alpha$ -methyl-3,4-dimethoxybenzyl group.
- 25 45. The nucleotide analogue of claim 37, wherein the unique label is attached through a cleavable linker to a 5-position of cytosine or thymine or to a 7-position of deaza-adenine or deaza-guanine.
- 30 46. The nucleotide analogue of claim 37, wherein the linker between the unique label and the nucleotide analogue is cleavable by a means selected from the group consisting of one or more of a physical

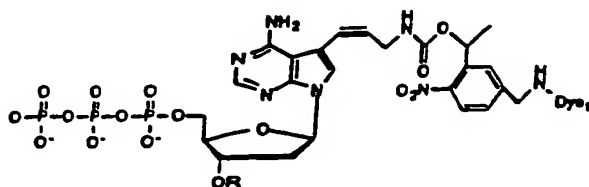
means, a chemical means, a physical chemical means, heat, and light.

5 47. The nucleotide analogue of claim 46, wherein the cleavable linker is a photocleavable linker which comprises a 2-nitrobenzyl moiety.

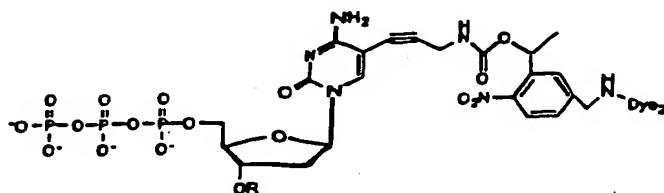
10 48. The nucleotide analogue of claim 37, wherein the cleavable chemical group used to cap the -OH group at the 3'-position of the deoxyribose is cleavable by a means selected from the group consisting of one or more of a physical means, a chemical means, a physical chemical means, heat, and light.

49. The nucleotide analogue of claim 37, wherein the nucleotide analogue is selected from the group consisting of:

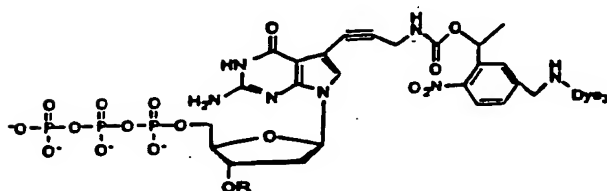
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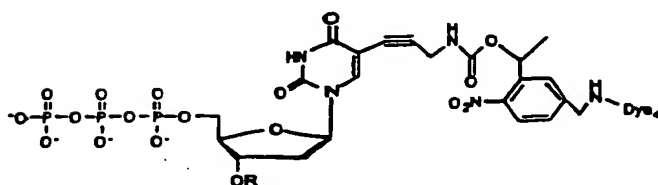
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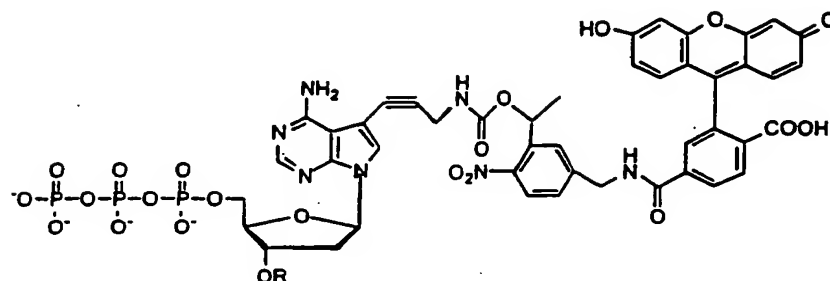
wherein Dye<sub>1</sub>, Dye<sub>2</sub>, Dye<sub>3</sub>, and Dye<sub>4</sub> are four different dye labels; and

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wherein R is a cleavable chemical group used to cap the -OH group at the 3'-position of the deoxyribose.

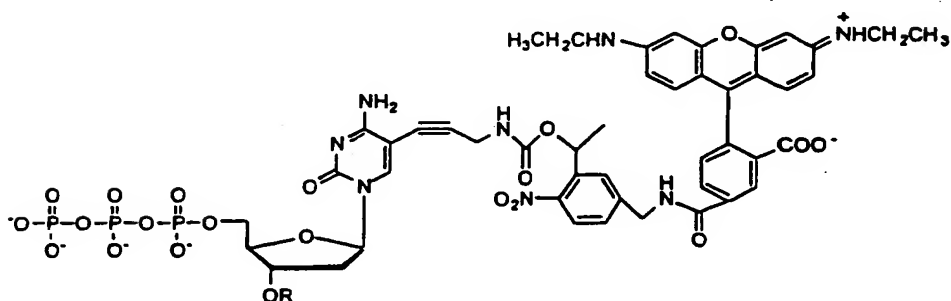
50. The nucleotide analogue of claim 49, wherein the nucleotide analogue is selected from the group consisting of:

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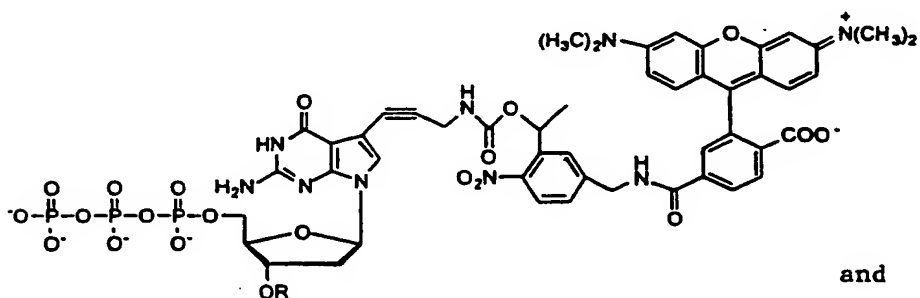


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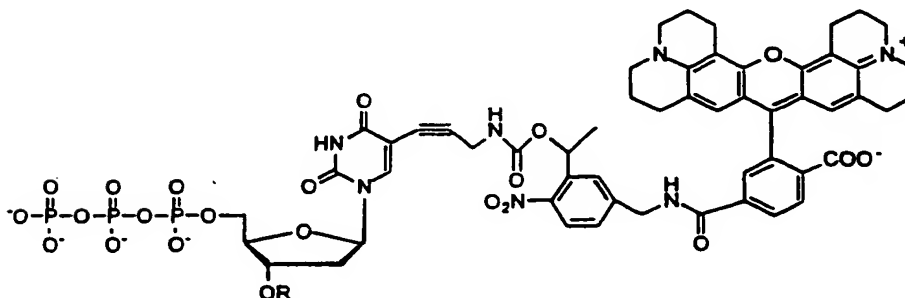


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and

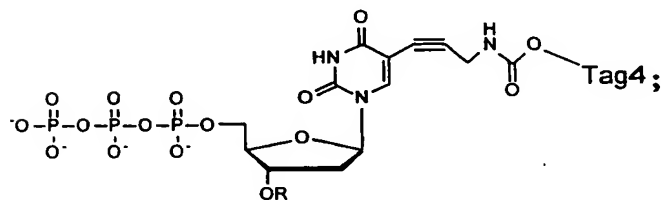
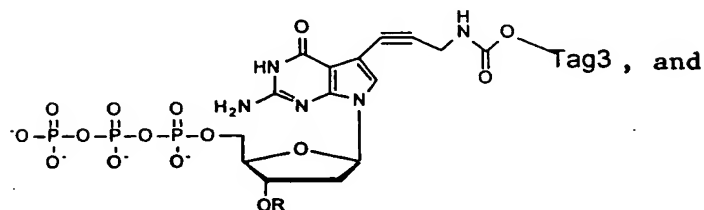
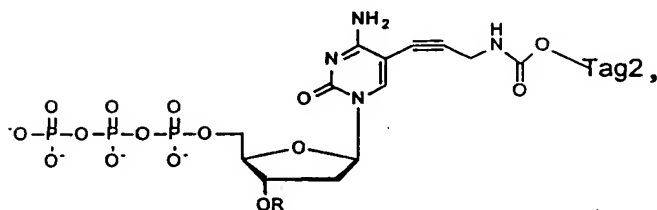
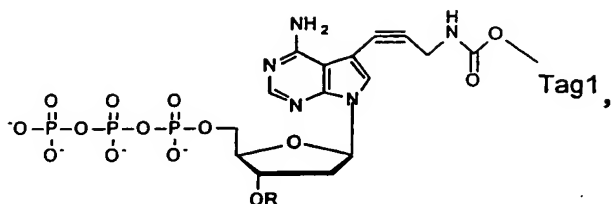
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wherein R is  $-\text{CH}_2\text{OCH}_3$  or  $-\text{CH}_2\text{CH}=\text{CH}_2$ .

51. The nucleotide analogue of claim 37, wherein the nucleotide analogue is selected from the group consisting of:



wherein Tag<sub>1</sub>, Tag<sub>2</sub>, Tag<sub>3</sub>, and Tag<sub>4</sub> are four different mass tag labels; and

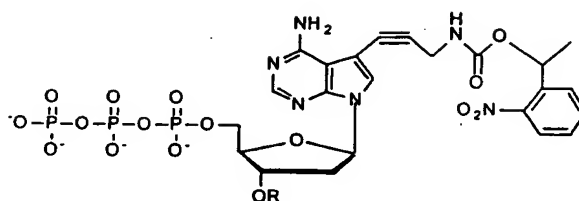
wherein R is a cleavable chemical group used to cap the -OH group at the 3'-position of the deoxyribose.



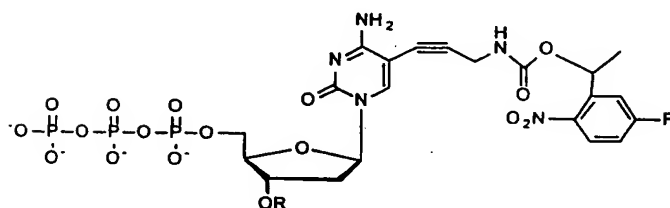
52. The nucleotide analogue of claim 51, wherein the nucleotide analogue is selected from the group consisting of:

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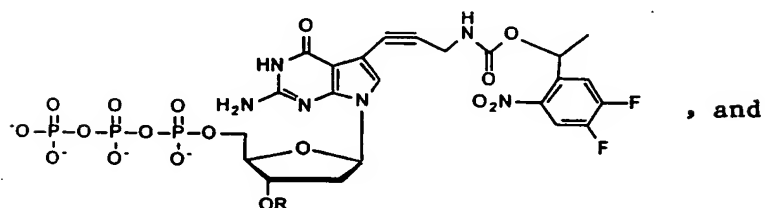
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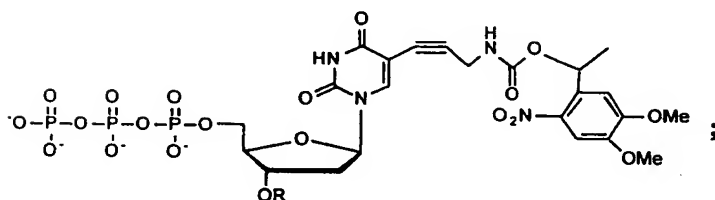
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wherein R is  $-\text{CH}_2\text{OCH}_3$  or  $-\text{CH}_2\text{CH}=\text{CH}_2$ .

53. Use of the nucleotide analogue of claim 37 for detection of single nucleotide polymorphisms, genetic mutation analysis, serial analysis of gene expression, gene expression analysis, identification in forensics, genetic disease association studies, DNA sequencing, genomic sequencing, translational analysis, or transcriptional analysis.
54. A parallel mass spectrometry system, which comprises a plurality of atmospheric pressure chemical ionization mass spectrometers for parallel analysis of a plurality of samples comprising mass tags.
55. The system of claim 54, wherein the mass spectrometers are quadrupole mass spectrometers or time-of-flight mass spectrometers.
56. The system of claim 54, wherein the mass spectrometers are contained in one device.
57. The system of claim 54 which further comprises two turbo-pumps, wherein one pump is used to generate a vacuum and a second pump is used to remove undesired elements.
58. The system of claim 54, which comprises at least three mass spectrometers.

59. The system of claim 54, wherein the mass tags have molecular weights between 150 daltons and 250 daltons.

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60. Use of the system of claim 54 for DNA sequencing analysis, detection of single nucleotide polymorphisms, genetic mutation analysis, serial analysis of gene expression, gene expression analysis, identification in forensics, genetic disease association studies, DNA sequencing, genomic sequencing, translational analysis, or transcriptional analysis.

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